ARTICLE

Synthesis of Pyrazolyl Thiobarbituric Acids and their Cytotoxic and Antimicrobial Evaluation

V. Rajashakar, K. Saisree, M. Sikender, S. Naveen, B. Madhava Reddy and V. Harinadha Babu✉

Asian Pub. Corp.

www.asianpubs.org

Asian Journal of Organic & Medicinal Chemistry

Volume: 1 Year: 2016
Issue: 3 Month: July-September
pp: 83–86
DOI: http://dx.doi.org/10.14233/ajomc.2016.AJOMC-P23

Received: 27 July 2016
Accepted: 8 August 2016
Published: 13 October 2016

ABSTRACT

Synthesis, cytotoxic and antimicrobial screening of novel thiobarbituric acid incorporated pyrazole derivatives were performed. Vilsmeier-Haack reaction of different phenyl hydrazones **1(a-e)** afforded pyrazole-4-carbaldehydes **2(a-e)** in good yields. Knoevenagel condensation of compounds **2(a-e)** with thiobarbituric acid gave a series of 5-ylidene derivatives **3(a-e)** in reasonable yields. The synthesized compounds were characterized with the help of IR, ^{1}H NMR and mass spectral data. The compounds were tested for cytotoxic activity against Vero, MCF-7 and HCT-116 cell lines. Among the tested compounds, 5-((3-(4-hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl)-methylene)-2-thioxodihydropyrimidine-4,6-(1*H*,5*H*)-dione **(3d)** was found to be most active molecule with the activity against both MCF-7 and HCT-116 cell lines with IC_{50} values of 14.0 µM and 18.12 µM. In antimicrobial screening, none of the compounds exhibited antibacterial activity.

KEYWORDS

Cytotoxic activity, Antimicrobial activity, Thiobarbituric acid, Pyrazoles.

INTRODUCTION

Pyrazoles are an interesting class of heterocyclic compounds with synthetic versatility and significant biological activities such as analgesic and anti-inflammatory [1-4], antidiabetic [5-7], antimicrobial [8-10], antiproliferative [11-14], *etc*. In recent years, a number of reports have been documented and few patents have been filed on pyrazole and pyrazoline derivatives as potent anticancer agents with B-Raf kinase inhibitor activity. The high profile NSAID, celecoxib contains a pyrazole nucleus having COX-2 inhibitory activity with few gasto intestinal side effects. However, there is an evidence of an increase in cardiovascular injury with its prolonged use and its anticancer properties are under clinical trials. Barbituric acid and thiobarbituric acid derivatives have anticonvulsant B [15], anti HIV [16], antibacterial [17], cyclin dependent kinase-2 and tyrosine kinase inhibitor activities [18]. 5-Benzylidene thiobarbituric acid derivatives and 5-benzylidene-4,6-pyrimidinediones [18] on the other hand, have been reported as novel

Author affiliations:

Department of Medicinal Chemistry, G. Pulla Reddy College of Pharmacy, Mehdipatnam, Hyderabad-500 028, India

✉To whom correspondence to be addressed:

E-mail: dr.harinathbabu@gmail.com

Available online at: http://ajomc.asianpubs.org

tyrosine kinase inhibitors and as well as antimicrobial compounds. It is known that a combination of different bioactive fragments with complimentary pharmacophoric functions and with different mechanism of action usually exhibit synergistic effects. Inspired by this information and also in continuation of our research on pyrazoles, in the present work, we made an attempt to combine these two scaffolds into a single molecule in order to obtain more potent antimicrobial and anticancer agents. Five novel pyrazolyl thiobarbituric acids **3(a-e)** were synthesized by condensing 1,3-diaryl pyrazole carbaldehydes with thiobarbituric acid. Vilsmeir-Haack reaction of phenyl hydrazones **1(a-e)** afforded pyrazole carbaldehyades **2(a-e)**. The structures of the synthesized compounds were confirmed on the basis of FTIR, ^{1}H NMR and mass spectral data. The synthesized compounds were screened for *in vitro* antibacterial and cytotoxic activities.

EXPERIMENTAL

All the solvents and chemicals used were of synthetic grade from SD fine chemicals Ltd., E. Merck, NR chemicals Ltd and Aldrich chemicals. Completion of the reactions was monitored by analytical thin layer chromatography (TLC) using E-Merck 0.25 mm silica gel plates. Visualization was accomplished with UV light (256 nm) and iodine chamber. Purification of synthesized compounds was done by re-crystallization process. The purity of the compounds was checked by a single spot in TLC. Melting points were determined in open capillary tubes using ANALAB melting point apparatus and are uncorrected. All the ^{1}H NMR spectra were recorded on AVANCE 300 MHz spectrometer using DMSO-d_6 as solvent and tetra methyl saline (TMS) as an internal standard. Chemical shift values are listed in δ scale. The IR spectra were recorded on Shimadzu FTIR spectrophotometer by using 1 % potassium bromide discs. Mass spectra of the compounds were recorded on Agilent 6430 triple quadruple LC-MS system and were given in mass units (*m/z*).

General procedure for synthesis of 3-aryl-1-phenyl-1*H*-pyrazole-4-carbaldehydes (2a-e): To an ice cold solution of DMF (0.1 mol), was added phosphorus oxychloride (0.013 mol) drop-wise and the temperature was maintained below 10 °C, since an exothermic reaction takes place. To the mixture, ice-cold solution of phenyl hydrazone (0.01 mol) was added in lots wise with stirring under ice cold condition. After the completion of the addition, the reaction mixture was stirred and refluxed at 60-70 °C for 12 h. Solution was cooled and poured into crushed ice with stirring and neutralized with $NaHCO_3$ solution. The solid obtained was filtered under suction and recrystallized from methanol.

General procedure for synthesis of 3-aryl-1-phenyl-1*H*-pyrazolyl-2-thiobarbituric acids (3a-e): A mixture of 3-aryl-1-phenyl-1*H*-pyrazol- 4-carbaldehyde (0.5 g, 2 mmol) and thiobarbituric acid (0.4 g, 2 m mol) in glacial acetic acid (20 mL) and 2-3 drops of piperidine was refluxed for 3-4 h. A solid was separated from the reaction mixture within 15-20 min and the refluxing was continued for 3-4 h in order to complete the reaction. The reaction mixture was cooled to room temperature, filtered and washed with ethanol to give the pure product (0.87 g, 90 % yield).

Biological activity

Cytotoxic activity: All the synthesized compounds were screened for MTT assay against Vero, (Normal cells) MCF-7 and HCT-116 cell lines and the test was performed at Natco Laboratories, Hyderabad. 1×10^4 cells/well were seeded in 100 µL DMEM supplemented with 10 % FBS in each well of 96 well microculture plates and incubated for 24 h at 37 °C in a CO_2 incubator. After incubation, cells were treated with test compounds **3(a-e)** at 100, 50, 25, 12.5, 6.25 µg/mL concentrations for 48 h. After 48 h of incubation, media was removed and to each well 10 µL of MTT (5 mg/mL) was added and the plates were further incubated for 4 h. Supernatant liquid from each well was carefully removed and formazon crystals were dissolved in 100 µL of DMSO and absorbance was measured at 540 nm wavelength.

Antibacterial activity: All the synthesized compounds were evaluated for antibacterial activity as per the standard procedures and the bacterial strains used were procured from National Chemical Laboratory, Pune. The activity was performed against two Gram-positive (*S. aureus, B. subtilis*) & two Gram-negative organisms *(P. aeruginosa, E. coli*) at concentrations of 1000, 500, 250 and 125 µg/mL using cup-plate method.

Physical and spectral data

5-[(1,3-Diphenyl-1*H*-pyrazol-4-yl)methylene]-2-thioxodihydropyrimidine-4,6-(1*H*,5*H*)-dione (3a): Cream coloured solid, Yield: 75 %, m.p.: 233-236 °C; IR (KBr, ν_{max}, cm^{-1}): 3339 (N-H str), 3036 (Ar C-H str), 1708 (C=O str), 1658 (C=O str), 1566 (C=N str); ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6):12.43 (s, 2H, NH of thiobarbituric acid), 9.84 (s, 1H, pyrazole), 8.18 (s, 1H, CH=), 7.4-7.9 (10H, Ar-H); Mass (*m/z*): 480.47 (M-H); Anal. calcd. for $C_{20}H_{14}N_4O_2S$: Calculated C, 64.16; H, 3.77; N, 14.96 %; found C, 64.13; H, 3.75; N, 14.94 %.

5-[{(3-4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-4yl}-methylene]-2-thioxodihydropyrimidine-4,6-(1*H*,5*H*)-dione (3b): White coloured solid, Yield: 78 %, m.p.: 224-228 °C; IR (KBr, ν_{max}, cm^{-1}): 3340 (N-H str), 3028 (Ar C-H str), 1708 (C=O str), 1660 (C=O str), 1558 (C=N str), 759 (C- Cl str); ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6), 12.45 (s, 2H, NH of thiobarbituric acid), 9.84 (s, 1H, pyrazole), 8.18 (s, 1H, CH=), 7.4-7.9 (9H, Ar-H); Mass (*m/z*): 409.1 (M+H); Anal. calcd. for $C_{20}H_{13}N_4O_2SCl$, Calculated C, 58.75; H, 3.20; N, 13.70 %; found C, 58.72; H, 3.18; N, 13.68 %.

5-[(1-Phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl)methylene]-2-thioxodihydropyrimidine-4,6-(1*H*,5*H*)-dione (3c): White coloured solid, Yield: 80 %, m.p.: 260-265 °C; IR (KBr, ν_{max}, cm^{-1}): 3349 (N-H str), 3022 (Ar C-H str), 2962 (C-H str of CH_3), 1708 (C=O str), 1658 (C=O str), 1565 (C=N str); ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6), 12.41 (s, 2H, NH of thiobarbituric acid), 9.84 (s, 1H, pyrazole), 8.18 (s, 1H, CH=), 7.4-7.9 (9H, Ar-H), 2.4-2.5 (3H, CH_3); Mass (*m/z*): 389.1 (M+H); Anal. calcd. for $C_{21}H_{16}N_4O_2S$, Calculated C, 64.93; H, 4.15; N, 14.42 %; found C, 64.92; H, 4.14; N, 14.40 %.

5-[{3-(4-Hydroxyphenyl)-1-phenyl-1*H*-pyrazol-4-yl}methylene]-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (3d): White coloured solid, Yield: 74 %, m.p.: 280-285 °C; IR (KBr, ν_{max}, cm^{-1}): 3348 (N-H str), 3340 (O-H str), 3028

(Ar C-H str), 1729 (C=O str), 1690 (C=O str), 1596 (C=N str); ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6), 12.41 (s, 2H, NH of thiobarbituric acid), 9.84 (s, 1H, pyrazole), 8.18 (s, 1H, CH=), 7.4-7.9 (9H, Ar-H), 5.4 (s, 1H, OH, exchangeable with D_2O); Mass (*m/z*): 391.1 (M+H); Anal. calcd. for $C_{20}H_{14}N_4O_3S$, Calculated C, 61.53; H, 3.61; N, 14.35 %; found C, 61.52; H, 3.59; N, 14.33 %.

5-[{3-(4-Naphalen-2-yl)-1-phenyl-1*H*-pyrazol-4-yl}-methylene]-2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione (3e): White coloured solid, Yield: 79 %, m.p.: 298-300 °C; IR (KBr, ν_{max}, cm^{-1}): 3337 (N-H str), 3047 (Ar C-H str), 1735 (C=O str), 1690 (C=O str), 1596 (C=N str); ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6), 12.41 (s, 2H, NH of thiobarbituric acid), 9.84 (s, 1H, pyrazole), 8.18 (s, 1H, CH=), 7.4-8.0 (12H, Ar-H); Mass (*m/z*): 425 (M+H); Anal. calcd. for $C_{25}H_{16}N_4O_2S$, Calculated C, 67.91; H, 3.80; N, 13.20 %; found C, 67.86; H, 3.68; N, 13.16 %.

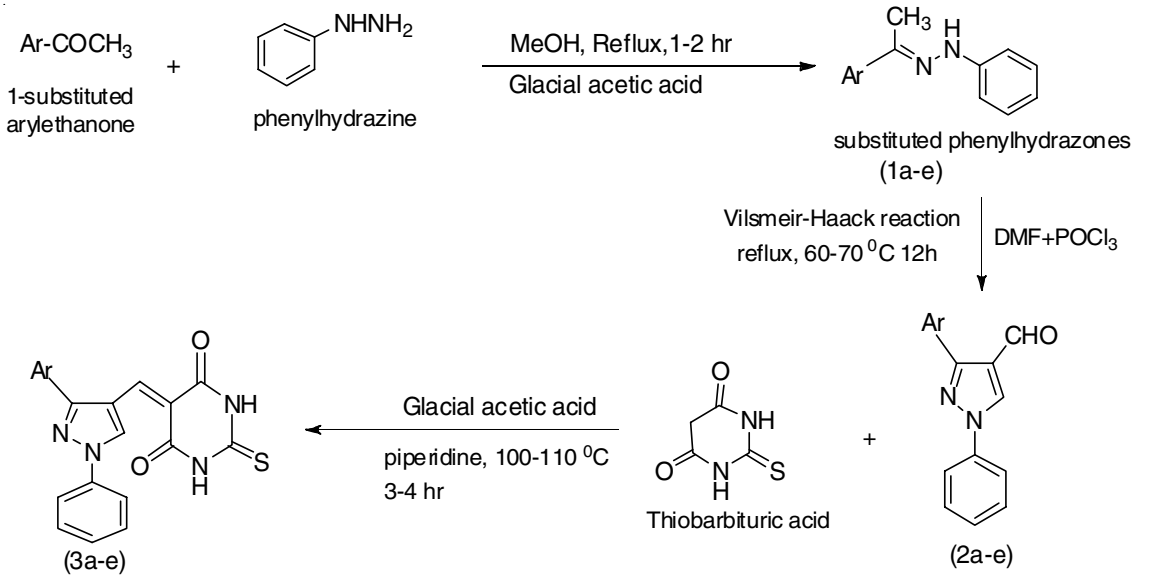
RESULTS AND DISCUSSION

The method for the synthesis of pyrazolyl thiobarbituric acids shown under **Scheme-I**. Different substituted phenyl hydrazones **(1a-e)** were subjected to Vilsmeier-Haack reaction under reflux at 60-70 °C for 12 h to give pyrazole-4 carbaldehydes **(2a-e)**. The purity of the compounds was confirmed by a single spot in TLC. In IR spectra, compounds showed carbonyl absorption around 1680-1670 cm^{-1} and C=N stretching around 1600 cm^{-1} which indicated the formation of pyrazole carbaldehydes. Moreover, compounds showed mass ion peaks of 100 % intensity corresponding to their molecular weights in mass spectra further confirmed the structures. The condensation of compounds **(2a-e)** with thiobarbituric acid in glacial acetic acid and few drops of piperidine under reflux for 3-4 h afforded pyrazolyl thiobarbituric acids in good yields. The confirmation of the structures of the synthesized compounds was done by FTIR, mass and ^{1}H NMR spectral data. Two carbonyl absorptions around 1710 cm^{-1} and 1660 cm^{-1} and N-H streching around 3350 cm^{-1} in IR spectra indicated the formation of the compounds. Further confirmation was obtained from ^{1}H NMR which showed a singlet integrating for two protons around δ 12.4 due to two NH protons, pyrazole proton appeared as singlet at δ 9.8 and aromatic protons and methine proton together appeared in the range of δ 7.4-8.0. Moreover, mass spectra of the compounds showed molecular ion peaks of 100 % intensity corresponding to their molecular weights.

Cytotoxic activity

***in vitro* Cytotoxic activity in cultured cells by MTT assay:** All the synthesized compounds were subjected for MTT assay against 3 cancer cell lines MCF-7, HCT-116 and Vero (Normal cells). The screening was carried out at 100, 50, 25, 12.5, 6.25 µg/mL concentrations. The IC_{50} values of the compounds were recorded and shown in Table-1. Compound **3d** exhibited highly potent activity against MCF-7 and HCT-116 cell lines in MTT assay at 14.02 µM and 18.12 µM concentrations. Similarly compound 3b also exhibited slightly less activity against both the cell lines. Other compounds displayed the activity in the range of 33.8 µM-82.0 µM concentrations.

Antibacterial activity: All the synthesized compounds were evaluated for antibacterial activity against four organisms at concentrations of 1000, 500, 250 and 125 µg/mL using cup-plate method against Gram-negative organisms (*P. aeruginosa*



Scheme-I

TABLE-1

Compound code	Cytotoxicity expressed as IC_{50} (μM) in cell lines		
	Vero	MCF-7	HCT-116
3a	173.4	54.1	60.8
3b	189.8	22.8	24.0
3c	> 400	82.0	66.0
3d	145.8	14.02	18.12
3e	132.0	33.8	42.0
Doxorubicin	3.1	1.544	1.964

IC_{50}: 50 % inhibitory concentration after 48 h of drug treatment and the values are average of three individual experiments.

and *E. coli*) and two Gram-positive organisms (*B. subtilis* and *S. aureus*).

None of the compounds have shown significant antimicrobial activity against the tested organisms even at 1000 μg/mL concentration.

Conclusion

Five novel pyrazolyl thiobarbituric acid derivatives were synthesized by combining pyrazole moiety with thiobarbituric acid scaffold and the synthesized compounds were characterized on the basis of physical and spectral data. In cytotoxic activity screening, compounds with hydroxyl and chloro substitutions on phenyl ring exhibited highly potent activity against MCF-7 and HCT-116 cell lines while the other three compounds were moderately active. None of the compounds exhibited significant antimicrobial activity. Further investigation of such thiobarbituric acid incorporated pyrazole derivatives could be interesting to get more selective anticancer agents.

ACKNOWLEDGEMENTS

The authors are grateful to the Management of G. Pulla Reddy College of Pharmacy, Hyderabad, India for providing research facilities. The authors also extended their sincere thanks to Natco Laboratories, Hyderabad, India for providing cytotoxic reports. Thanks are also due to University of Hyderabad, India for providing mass and NMR spectral data.

REFERENCES

1. K. Mogilaiah, K. Vidya and S. Kavitha, *Indian J. Chem.*, **48B**, 282 (2009).
2. D.R. Nagargoje, A.R. Ghawalkar and G.R. Jadhav, *Indian J. Hetereocycl. Chem.*, **18**, 53 (2008).
3. K.S. Swetha, R. Parameshwar, B.M. Reddy and V.H. Babu, *Med. Chem. Res.*, **22**, 4886 (2013); http://dx.doi.org/10.1007/s00044-013-0500-0.
4. A.M. Youssef, M.S. White, E.B. Villanueva, I.M. El-Ashmawy and A. Klegeris, *Bioorg. Med. Chem.*, **18**, 2019 (2010); http://dx.doi.org/10.1016/j.bmc.2010.01.021.
5. O. Prakash, R. Pundeera and P. Ranjana, *Indian J. Hetereocycl. Chem.*, **48**, 563 (2009).
6. P.A. Datar and S.R. Jadhav, *Lett. Drug Des. Discov.*, **11**, 686 (2013); http://dx.doi.org/10.2174/15701808106661311132123 54.
7. G.R. Krutgen, A. Forsch and U. Klemn, *Chem. Abstr.*, **31**, 649 (1981).
8. S.N. Thore and A.K. Gupta, *Indian J. Hetereocycl. Chem.*, **19**, 329 (2010).
9. A.A. Bekhit, H.M. Ashour, A.D. Bekhit and S. Bekhit, *Med. Chem.*, **5**, 103 (2009); http://dx.doi.org/10.2174/157340609787582936.
10. E.A. Musad, R. Mohamed, B.A. Saeed, B.S. Vishwanath and K.M. Lokanatha Rai, *Bioorg. Med. Chem. Lett.*, **21**, 3536 (2011); http://dx.doi.org/10.1016/j.bmcl.2011.04.142.
11. P.-C. Lv, H.-Q. Li, J. Sun, Y. Zhou and H.-L. Zhu, *Bioorg. Med. Chem.*, **18**, 4606 (2010); http://dx.doi.org/10.1016/j.bmc.2010.05.034.
12. P. Pevarello, M.G. Brasca, R. Amici, P. Orsini, G. Traquandi, L. Corti, C. Piutti, P. Sansonna, M. Villa, B.S. Pierce, M. Pulici, P. Giordano, K. Martina, E.L. Fritzen, R.A. Nugent, E. Casale, A. Cameron, M. Ciomei, F. Roletto, A. Isacchi, G.P. Fogliatto, E. Pesenti, W. Pastori, A. Marsiglio, K.L. Leach, P.M. Clare, F. Fiorentini, M. Varasi, A. Vulpetti and M.A. Warpehoski, *J. Med. Chem.*, **47**, 3367 (2004); http://dx.doi.org/10.1021/jm031145u.
13. R. Lin, G. Chiu, Y. Yu, P.J. Connolly, S. Li, Y. Lu, M. Adams, A.R. Fuentes-Pesquera, S.L. Emanuel and L.M. Greenberger, *Bioorg. Med. Chem. Lett.*, **17**, 4557 (2007); http://dx.doi.org/10.1016/j.bmcl.2007.05.092.
14. M.S. Christodoulou, S. Liekens, K.M. Kasiotis and S.A. Haroutounian, *Bioorg. Med. Chem.*, **18**, 4338 (2010); http://dx.doi.org/10.1016/j.bmc.2010.04.076.
15. A. Agarwal, S. Lata, K.K. Saxena, V.K. Srivastava and A. Kumar, *J. Med. Chem.*, **41**, 1223 (2006); http://dx.doi.org/10.1016/j.ejmech.2006.03.029.
16. R. Costi, R.D. Santo, M. Artico, S. Massa, R. Ragno, R. Loddo, M. La Colla, E. Tramontano, P. La Colla and A. Pani, *Bioorg. Med. Chem.*, **12**, 199 (2004); http://dx.doi.org/10.1016/j.bmc.2003.10.005.
17. T. Tomasic, N. Zidar, V. Rupnik, A. Kovac, D. Blanot, S. Gobec, D. Kikelj and L.P. Mašic, *Bioorg. Med. Chem. Lett.*, **19**, 153 (2009); http://dx.doi.org/10.1016/j.bmcl.2008.10.129.
18. Z. Chen, D. Cai, D. Mou, Q. Yan, Y. Sun, W. Pan, Y. Wan, H. Song and W. Yi, *Bioorg. Med. Chem. Lett.*, **22**, 3279 (2014); http://dx.doi.org/10.1016/j.bmc.2014.04.060.